Correlation between the anesthetic potency of local anesthetics and their binding ability to a model membrane

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Abstract: The interaction between various local anesthetics and the phospholipid membrane was examined by 1H-NMR (nuclear magnetic resonance) spectroscopy. By examining the chemical shift value in order to measure the extent of proximity of various local anesthetics to the membrane, it was determined that tetracaine (10.7Hz) was closest to the membrane, followed in descending order of proximity by dibucaine (8.8Hz), bupivacaine (4.4Hz), propitocaine (4.4Hz), and lidocaine (3.5Hz). Procaine and cocaine did not affect the chemical shift value. In addition, we studied the interaction of local anesthetics with the membrane by examining the broadening of the half-width, and determined that tetracaine (12.2Hz) bound closest to the membrane, followed in descending order of proximity by dibucaine (11.0Hz), bupivacaine (9.6Hz), propitocaine (9.0Hz), lidocaine (8.8Hz), procaine (8.0Hz) and cocaine (7.9Hz). In the present study, the binding ability of local anesthetics to the phospholipid membrane was found to be directly in parallel with the potency and toxicity of the anesthetic.

Key words: Local anesthetics, NMR, Biomembrane, Binding ability, Site of action

Introduction

The site of local anesthetic action is well known to be the sodium channel. However, several questions have been raised in response to Hille’s theory that specific receptors exist for local anesthetics [1]. For example, the effective concentration of drugs for which expression of the pharmacological effect depends on specific receptors, such as morphine, acetylcholine, and tetrodotoxin, is on the order of nanomoles, but that of local anesthetics is on the order of millimoles [2]. This difference is about 1 million-fold. It is difficult to accept that specific receptors exist for chemicals that are effective only at such high concentrations.

Also, according to Hille, local anesthetics pass through the biomembrane, and enter sodium channels inside [1]. However, there is no scientific evidence demonstrating that local anesthetics pass through the biomembrane. The results of a study we conducted using NMR showed that when local anesthetics were left for 1 month at 37°C in an environment in which the pH was raised to 10, thereby increasing the amount of basic local anesthetic 1000 times, the anesthetic did not pass through the membrane and instead remained on the surface [3].

Researchers do not dispute that the sodium channel is the site of local anesthetic action. However, local anesthetics can inhibit the influx of the sodium ion without entering the channel. For example, local anesthetics can inhibit the passage of the sodium ion by binding to the biomembrane that surrounds the sodium channel, thereby altering the conformation of channel proteins and expressing their pharmacological effect [4].

Previously, we synthesized new ester derivatives of lidocaine with a longer duration of action than that of lidocaine, and the interaction between the derivatives and phospholipid membrane was examined by NMR spectroscopy [5]. The duration of the ester derivatives was found to be three times longer than that of lidocaine [6], because the derivatives bound to the membrane at two location [4,5]. Therefore, in the present study, NMR was utilized to measure the binding ability of each local anesthetic with the phospholipid membrane in order to compare the pharmacological characteristics (potency and toxicity) of the anesthetics.

Materials and methods

The interaction between 110mM of local anesthetics hydrochloride (lidocaine, propitocaine, bupivacaine,
dibucaine, tetracaine, procaine, and cocaine, pH 6.2–6.8) and the phospholipid model membrane (66 mM of egg yolk lecithin) was examined by 1H-NMR. Lecithin dispersions were obtained by evaporating lecithin solution (1-α-phosphatidyl-choline type III-E from egg yolk, 100 mg·mL⁻¹ in hexane) to dryness, dissolving the residue in D₂O, and subjecting the resulting coarse dispersion to an ultrasonic disintegrator (W-220, Heat System-Ultrasonic, Inc., New York, USA) for 1 h in an ice-cold vessel under a nitrogen atmosphere. ¹H-NMR spectra were measured in a 5-mm tube at 27°C using a JNM-EX-400 spectrometer (JEOL, Tokyo, Japan). Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as the internal standard (0.0 ppm).

Based on the results of previous studies [3–5], it was thought that the binding between local anesthetics and the membrane occurs in the oxygen atom on the phosphate adjacent to the choline methyl, which is the hydrophilic region on the external surface of the membrane. Therefore, a hydrogen atom that exists most proximally to the oxygen atom, the choline methyl signal, was used for ¹H-NMR to measure the chemical shift and the broadening of half-width (peak width at half maximum height) of hydrogen atoms signal.

**Results**

The ¹H-NMR spectrum of the phospholipid membrane in D₂O is shown in Fig. 1A. The most prominent signals, identified as choline methyl protons, methyl protons, and methylene protons, showed a chemical shift at 3.26 ppm (1302.4 Hz), 0.85 ppm (340.3 Hz), and 1.31 ppm (523.8 Hz) from DSS, respectively. When lidocaine hydrochloride (110 mM) was added to the solution, the chemical shift of the methylene and methyl signals showed no change, but the choline methyl signal shifted 3.5 Hz to upfield (1302.4 Hz → 1298.9 Hz, Fig. 1B) from the internal reference (DSS). This provides evidence of the interaction between anesthetics and lecithin vesicles on the membrane surface. A similar upfield shift was observed after the addition of propitocaine (4.4 Hz), bupivacaine (4.4 Hz), dibucaine (8.8 Hz), and tetracaine (10.7 Hz), whereas no chemical shift was observed after the addition of procaine and cocaine. These results are attributable to the change in electrical environment of the polar part of the membrane surface resulting from the approach of the incorporated anesthetics. Furthermore, the degree of the chemical shift probably depends on the extent of proximity between the phospholipid membrane and anesthetics (Fig. 2).

![Fig. 1. A Nuclear magnetic resonance (NMR) spectrum of phospholipid membrane (66 mM). B NMR spectrum of phospholipid membrane with lidocaine (110 mM). DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate](image-url)